

## GROWTH HORMONE SECRETAGOGUES

### FIELD OF THE INVENTION

- 5           The invention relates to compounds, which are useful for administration to a mammal thereby elevating the plasma level of growth hormone.

### BACKGROUND OF THE INVENTION

#### (a) Description of Prior Art

- 10           Growth hormone (GH) or somatotropin, secreted by the pituitary gland constitute a family of hormones which biological activity is fundamental for the linear growth of a young organism but also for the maintenance of the integrity at its adult state. GH acts directly or indirectly on the peripheral organs by stimulating the synthesis of growth factors (insulin-like growth factor-I or IGF-I) or of their receptors (epidermal growth factor or
- 15 EGF). The direct action of GH is of the type referred to as anti-insulinic, which favors the lipolysis at the level of adipose tissues. Through its action on IGF-I (somatomedin C) synthesis and secretion, GH stimulates the growth of the cartilage and the bones (structural growth), the protein synthesis and the cellular proliferation in multiple peripheral organs, including muscles and the skin. Through its biological activity, GH participates within
- 20 adults at the maintenance of a protein anabolism state, and plays a primary role in the tissue regeneration phenomenon after a trauma.

- The decrease of GH secretion with the age, demonstrated in humans and animals, favors a metabolic shift towards catabolism which initiates or participates to the ageing of an organism. The loss in muscle mass, the accumulation of adipose tissues, the bone
- 25 demineralization, the loss of tissue regeneration capacity after an injury, which are observed in elderly, correlate with the decrease in the secretion of GH.

          GH is thus a physiological anabolic agent absolutely necessary for the linear growth of children and which controls the protein metabolism in adults.

- Growth hormone (GH) secretion is regulated by two hypothalamic peptides:
- 30 GH-releasing hormone (GHRH), which exerts stimulatory effect on GH release and somatostatin which exhibits an inhibitory influence. In the last few years, several investigators have demonstrated that GH secretion can also be stimulated by synthetic oligopeptides termed GH-releasing peptides (GHRP) such as hexarelin and various hexarelin analogs (Ghigo et al., European Journal of Endocrinology, 136, 445-460, 1997).
- 35 These compounds act through a mechanism which is distinct from that of GHRH (C.Y. Bowers, in "Xenobiotic Growth Hormone Secretagogues", Eds. B.Bercu and R.F. Walker, Pg. 9-28, Springer-Verlag, New York 1996) and by interaction with specific receptors

localized in the hypothalamus and pituitary gland ((a) G. Muccioli et al., Journal of Endocrinology, 157, 99-106, 1998; (b) G. Muccioli, "Tissue Distribution of GHRP Receptors in Humans", Abstracts IV European Congress of Endocrinology, Sevilla, Spain, 1998). Recently it was demonstrated that GHRP receptors are present not only in the hypothalamo-pituitary system but even in various human tissues not generally associated with GH release (G. Muccioli et al., see above (a)).

- GHRPs and their antagonists are described, for example, in the following publications: C.Y. Bowers, supra, R. Deghenghi, "Growth Hormone Releasing Peptides", ibidem, 1996, pg. 85-102; R. Deghenghi et al., "Small Peptides as Potent Releasers of Growth Hormone", J. Ped. End. Metab., 8, pg. 311-313, 1996; R. Deghenghi, "The Development of Impervious Peptides as Growth Hormone Secretagogues", Acta Paediatr. Suppl., 423, pg. 85-87, 1997; K. Veeraranagan et al., "Growth Hormone Releasing Peptides (GHRP) Binding to Porcine Anterior Pituitary and Hypothalamic Membranes", Life Sci., 50, Pg. 1149-1155, 1992; and T.C. Somers et al., "Low Molecular Weight Peptidomimetic Growth Hormone Secretagogues, WO 96/15148 (May 23, 1996).

The human GH has been produced by genetic engineering for about ten years. Until recently most of the uses of GH were concerned with growth delay in children and now the uses of GH in adults are studied. The pharmacological uses of GH, GHRPs and growth hormone secretagogues and may be classified in the following three major categories.

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(b) Children growth

Treatments with recombinant human growth hormone have been shown to stimulate growth in children with pituitary dwarfism, renal insufficiencies, Turner's syndrome and short stature. Recombinant human GH is presently commercialized in Europe and in the United States for children's growth retardation caused by a GH deficiency and for children's renal insufficiencies. The other uses are under clinical trial investigation.

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(c) Long term treatment for adults and elderly patients

A decrease in GH secretion causes changes in body composition during aging.

- Preliminary studies of one-year treatment with recombinant human GH reported an increase in the muscle mass and in the thickness of skin, a decrease in fat mass with a slight increase in bone density in a population of aged patients. With respect to osteoporosis, recent studies suggest that recombinant human GH does not increase bone mineralization but it is suggested that it may prevent bone demineralization in post-menopausal women. Further studies are currently underway to demonstrate this theory.

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(d) Short term treatment in adults and elderly patients

In preclinical and clinical studies, growth hormone has been shown to stimulate protein anabolism and healing in cases of burn, AIDS and cancer, in wound and bone healing.

- 5 GH, GHRPs and growth hormone secretagogues are also intended for veterinary pharmacological uses. GH, GHRPs and growth hormone secretagogues stimulate growth in pigs during its fattening period by favoring the deposition of muscle tissues instead of adipose tissues and increase milk production in cows, and this without any undesired side effects which would endanger the health of the animals and without any residue in the meat or milk being produced. The bovine somatotropin (BST) is presently commercialized in the United States.

Most of the clinical studies presently undertaken were conducted with recombinant GH. The GHRPs and growth hormone secretagogues are considered as a second generation product destined to replace in the near future the uses of GH in most instances.

- 15 Accordingly, the use of GHRPs and growth hormone secretagogues present a number of advantages over the use of GH per se.

Therefore, there is a need for compounds which, when administered to a mammal, act as growth hormone secretagogues.

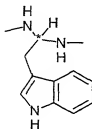
20 SUMMARY OF THE INVENTION

The present invention relates to new compounds which act as growth hormone secretagogues and, in general, to a method for elevating the plasma level of growth hormone in a mammal by administering thereto one or more of the compounds according to the invention. The invention also relates to methods for the treatment of growth hormone secretion deficiency, for promoting wound healing, recovery from surgery or recovery from debilitating illnesses, by administering to a mammal one of these compounds in a therapeutically effective amount.

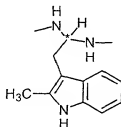
DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

- 30 In this description, the following abbreviations are used: D is the dextro enantiomer, GH is growth hormone, Boc is tert-butyloxycarbonyl, Z is benzyloxycarbonyl, N-Me is N-methyl, Pip is 4-amino-piperidine-4-carboxylate, Inip is isonipecotyl, i.e. piperidine-4-carboxylate, Aib is  $\alpha$ -amino isobutyryl, Nal is  $\beta$ -naphthylalanine, Mrp is 2-Methyl-Trp, and Ala, Lys, Phe, Trp, His, Thr, Cys, Tyr, Leu, Gly, Ser, Pro, Glu, Arg, Val and Gln are the amino acids alanine, lysine, phenylalanine, tryptophan, histidine, threonine,
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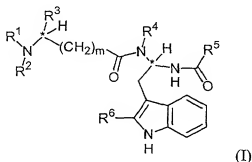
cysteine, tyrosine, leucine, glycine, serine, proline, glutamic acid, arginine, valine and glutamine, respectively. Furthermore gTrp is a group of the formula



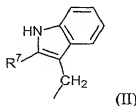
and gMrp a group of the formula



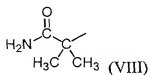
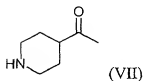
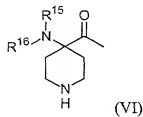
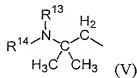
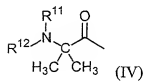
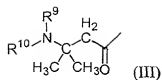
wherein \* means a carbon atom which, when a chiral carbon atom, has a R or S<sup>1</sup> configuration. The compounds of the invention are of the general formula I:



wherein \* means a carbon atom which, when a chiral carbon atom, has a R or S configuration, one of R<sup>1</sup> and R<sup>3</sup> is an hydrogen atom and the other is a group of formula II



R<sup>2</sup> is a hydrogen atom, a linear or branched C<sub>1</sub>-C<sub>6</sub> alkyl group, an aryl group, a heterocyclic group, a cycloalkyl group, a (CH<sub>2</sub>)<sub>n</sub>-aryl group, a (CH<sub>2</sub>)<sub>n</sub>-heterocyclic group, a (CH<sub>2</sub>)<sub>n</sub>-cycloalkyl group, a methylsulfonyl group, a phenylsulfonyl group, a C(O)R<sup>8</sup> group or a group according to one or formulas III to VIII below:



$R^4$  is a hydrogen atom or a linear or branched  $C_1$ - $C_4$ -alkyl group,  $R^5$  is a hydrogen atom, a linear or branched  $C_1$ - $C_4$  alkyl group, a  $(CH_2)_n$ -aryl group, a  $(CH_2)_n$ -heterocycle group, a  $(CH_2)_n$ -cycloalkyl group or an amino group,  $R^6$  and  $R^7$  are independently from each other a hydrogen atom or a linear or branched  $C_1$ - $C_4$ -alkyl group,  $R^8$  is a linear or branched

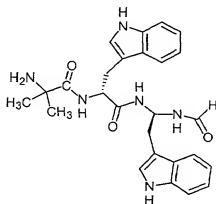
C<sub>1</sub>-C<sub>6</sub>-alkyl group, R<sup>9</sup>, R<sup>10</sup>, R<sup>11</sup>, R<sup>12</sup>, R<sup>13</sup>, R<sup>14</sup>, R<sup>15</sup>, and R<sup>16</sup> are independently from each other a hydrogen atom or a linear or branched C<sub>1</sub>-C<sub>4</sub>-alkyl group, m is 0, 1 or 2 and n is 1 or 2.

A preferred embodiment of the invention are compounds wherein R<sup>2</sup> is hydrogen, R<sup>3</sup> is a group of formula II and m is 0. Particularly preferred are compounds, wherein linear or branched C<sub>1</sub>-C<sub>4</sub> alkyl is methyl, linear or branched C<sub>1</sub>-C<sub>6</sub> alkyl is methyl, ethyl or i-butyl, aryl is phenyl or naphthyl, cycloalkyl is cyclohexyl and the heterocyclic group is a 4-piperidinyl or 3-pyrrolyl group.

Specifically preferred compounds of the invention include the following:

10 H-Aib-D-Trp-D-gTrp-CHO:

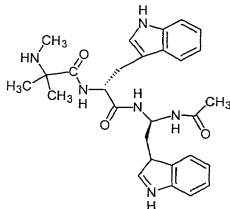
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N-Me-Aib-D-Trp-D-gTrp-C(O)CH<sub>3</sub>:

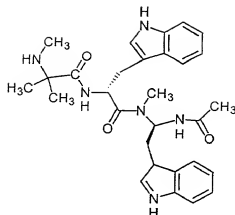
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N-Me-Aib-D-Trp-N-Me-D-gTrp-C(O)CH<sub>3</sub>:

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In accordance with the present invention, it has been found that the compounds of the invention are useful for elevating the plasma level of growth hormone in a mammal. Furthermore the compounds of the present invention are useful for the treatment of growth hormone secretion deficiency, growth retardation in child and metabolic disorders associated with growth hormone secretion deficiency, in particular in aged subjects.

Pharmaceutically acceptable salts of these compounds can be also used, if desired. Such salts include organic or inorganic addition salts, such as hydrochloride, hydrobromide, phosphate, sulfate, acetate, succinate, ascorbate, tartrate, gluconate, benzoate, malate, fumarate, stearate or pamoate salts.

Pharmaceutical compositions of the invention are useful for elevating the plasma level of growth hormone in a mammal, including a human, as well for the treatment of growth hormone secretion deficiency, growth retardation in child and metabolic disorders associated with growth hormone secretion deficiency, in particular in aged subjects. Such pharmaceutical compositions can comprise a compound according to the present invention or a pharmaceutically acceptable salt thereof, or combinations of compounds according to the present invention or pharmaceutically acceptable salts thereof, optionally in admixture with a carrier, excipient, vehicle, diluent, matrix, or delayed release coating. Examples of such carriers, excipients, vehicles, and diluents, can be found in Remington's Pharmaceutical Sciences, 18th Edition, A.R. Gennaro, Ed., Mack Publishing Company, Easton, PA, 1990.

The pharmaceutical compositions of the invention can comprise an additional growth hormone secretagogue. Examples for suitable additional growth hormone secretagogues are Ghrelin (cf. M. Kojima et al., Nature, 402 (1999), 656-660), GHRP-1, GHRP-2 and GHRP-6.

Ghrelin: Gly-Ser-Ser(O-n-octanoyl)-Phe-Leu-Ser-Pro-Glu-His-Gln-Arg-Val-Gln-Gln-Arg-Lys-Glu-Ser-Lys-Lys-Pro-Pro-Ala-Lys-Leu-Gln-Pro-Arg

GHRP-1: Ala-His-D-β-Nal-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>

GHRP-2: D-Ala-D-β-Nal-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>

5 GHRP-6: His-D-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>

Any of the compounds according to the present invention can be formulated by the skilled in the art to provide medicaments which are suitable for parenteral, buccal, rectal, vaginal, transdermal, pulmonary or oral routes of administration.

The type of formulation of the medicament containing the compound can be selected  
10 according to the desired rate of delivery. For example, if the compounds are to be rapidly delivered, the nasal or intravenous route is preferred.

The medicaments can be administered to mammals, including humans, at a therapeutically effective dose which can be easily determined by one of skill in the art and which can vary according to the species, age, sex and weight of the treated patient or subject  
15 as well the route of administration. The exact level can be easily determined empirically.

### EXAMPLES

The following examples illustrate the efficacy of the most preferred compounds used in the treatment of this invention.

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Example 1: H-Aib-D-Trp-D-gTrp-CHO

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Total synthesis (percentages represent yields obtained in the synthesis as described below):

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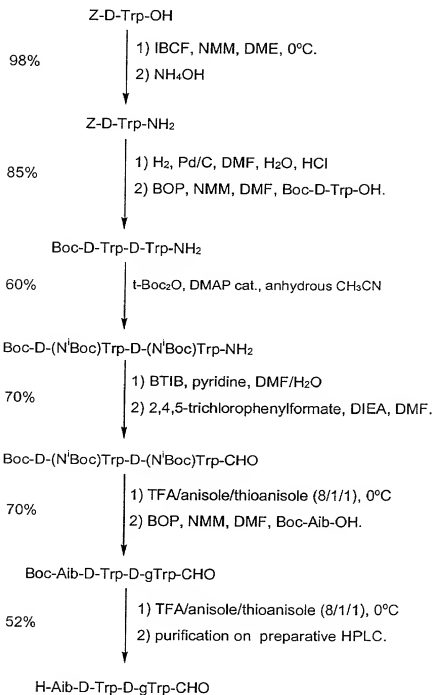
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13%



### Z-D-Trp-NH<sub>2</sub>

Z-D-Trp-OH (8.9g; 26mmol; 1eq.) was dissolved in DME (25ml) and placed in an ice water bath to 0°C. NMM (3.5ml; 1.2eq.), IBCF (4.1ml; 1.2eq.) and ammonia solution 28% (8.9ml; 5eq.) were added successively. The mixture was diluted with water (100ml), and the product Z-D-Trp-NH<sub>2</sub> precipitated. It was filtered and dried *in vacuo* to afford 8.58g of a white solid.

Yield = 98%.

C<sub>19</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub>, 337 g.mol<sup>-1</sup>.

Rf = 0.46 {Chloroform/Methanol/ Acetic Acid (180/10/5)}.

<sup>1</sup>H NMR (250 MHz, DMSO-d<sub>6</sub>) : δ 2.9 (dd, 1H, H<sub>β</sub>, J<sub>ββ'</sub> = 14.5Hz; J<sub>βα</sub> = 9.8Hz); 3.1 (dd, 1H, H<sub>β</sub>, J<sub>ββ'</sub> = 14.5Hz; J<sub>β'α</sub> = 4.3Hz); 4.2 (sextuplet, 1H, H<sub>α</sub>); 4.95 (s, 2H, CH<sub>2</sub> (Z)); 6.9-7.4 (m, 11H); 7.5 (s, 1H, H<sup>2</sup>); 7.65 (d, 1H, J = 7.7Hz); 10.8 (s, 1H, N<sup>1</sup>H).

Mass Spectrometry (Electrospray), m/z 338 [M+H]<sup>+</sup>, 360 [M+Na]<sup>+</sup>, 675 [2M+H]<sup>+</sup>, 697 [2M+Na]<sup>+</sup>.

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### Boc-D-Trp-D-Trp-NH<sub>2</sub>

Z-D-Trp-NH<sub>2</sub> (3g; 8.9mmol; 1eq.) was dissolved in DMF (100ml). HCl 36% (845μl; 1.1 eq.), water (2ml) and palladium on activated charcoal (95mg, 0.1eq.) were added to the stirred mixture. The solution was bubbled under hydrogen for 24 hr. When the reaction went to completion, the palladium was filtered on celite. The solvent was removed *in vacuo* to afford HCl, H-D-Trp-NH<sub>2</sub> as a colorless oil.

In 10ml of DMF, HCl, H-D-Trp-NH<sub>2</sub> (8.9mmol; 1eq.), Boc-D-Trp-OH (2.98g; 9.8mmol; 1.1eq.), NMM (2.26ml; 2.1eq.) and BOP (4.33g; 1.1eq.) were added successively. After 1 hr, the mixture was diluted with ethyl acetate (100ml) and washed with saturated aqueous sodium hydrogen carbonate (200ml), aqueous potassium hydrogen sulfate (200ml, 1M), and saturated aqueous sodium chloride (100ml). The organic layer was dried over sodium sulfate, filtered and the solvent removed *in vacuo* to afford 4.35g of Boc-D-Trp-D-Trp-NH<sub>2</sub> as a white solid.

Yield = 85%.

<sup>30</sup> C<sub>27</sub>H<sub>31</sub>N<sub>5</sub>O<sub>4</sub>, 489 g.mol<sup>-1</sup>.

Rf = 0.48 {Chloroform/Methanol/Acetic Acid (85/10/5)}.

<sup>1</sup>H NMR (200 MHz, DMSO-d<sub>6</sub>) : δ 1.28 (s, 9H, Boc); 2.75-3.36 (m, 4H, 2 (CH<sub>2</sub>)<sub>β</sub>; 4.14 (m, 1H, CH<sub>α</sub>); 4.52 (m, 1H, CH<sub>α</sub>); 6.83-7.84 (m, 14H, 2 indoles (10H), NH<sub>2</sub>, NH (urethane) and NH (amide)); 10.82 (d, 1H, J = 2Hz, N<sup>1</sup>H); 10.85 (d, 1H, J = 2Hz, N<sup>1</sup>H).

Mass Spectrometry (Electrospray), m/z 490 [M+H]<sup>+</sup>, 512 [M+Na]<sup>+</sup>, 979 [2M+H]<sup>+</sup>.

Boc-D-(N<sup>i</sup>Boc)Trp-D-(N<sup>i</sup>Boc)Trp-NH<sub>2</sub>

Boc-D-Trp-D-Trp-NH<sub>2</sub> (3g; 6.13mmol; 1 eq.) was dissolved in acetonitrile (25ml). To this solution, di-tert-butyl-dicarbonate (3.4g; 2.5eq.) and 4-dimethylaminopyridine (150mg; 0.2eq.) were successively added. After 1 hr, the mixture was diluted with ethyl acetate (100ml) and washed with saturated aqueous sodium hydrogen carbonate (200ml), aqueous potassium hydrogen sulfate (200ml, 1M), and saturated aqueous sodium chloride (200ml). The organic layer was dried over sodium sulfate, filtered and the solvent removed *in vacuo*. The residue was purified by flash chromatography on silica gel eluting with ethyl acetate/hexane {5/5} to afford 2.53g of Boc-D-(N<sup>i</sup>Boc)Trp-D-(N<sup>i</sup>Boc)Trp-NH<sub>2</sub> as a white

10 solid.

Yield = 60%.

C<sub>37</sub>H<sub>47</sub>N<sub>5</sub>O<sub>8</sub>, 689 g.mol<sup>-1</sup>.

R<sub>f</sub> = 0.23 {ethyl acetate/hexane (5/5)}.

<sup>1</sup>H NMR (200 MHz, DMSO-d<sub>6</sub>) : δ 1.25 (s, 9H, Boc); 1.58 (s, 9H, Boc); 1.61 (s, 15 9H, Boc); 2.75-3.4 (m, 4H, 2 (CH<sub>2</sub>)<sub>β</sub>); 4.2 (m, 1H, CH<sub>α</sub>); 4.6 (m, 1H, CH<sub>α</sub>); 7.06-8 (m, 14H, 2 indoles (10H), NH (urethane), NH and NH<sub>2</sub> (amides)).

Mass Spectrometry (Electrospray), m/z 690 [M+H]<sup>+</sup>, 712 [M+Na]<sup>+</sup>, 1379 [2M+H]<sup>+</sup>, 1401 [2M+Na]<sup>+</sup>.

20 Boc-D-(N<sup>i</sup>Boc)Trp-D-g(N<sup>i</sup>Boc)Trp-H

Boc-D-(N<sup>i</sup>Boc)Trp-D-(N<sup>i</sup>Boc)Trp-NH<sub>2</sub> (3g; 4.3mmol; 1eq.) was dissolved in the mixture DMF / water (18ml / 7ml). Then, pyridine (772μl; 2.2eq.) and Bis(Trifluoroacetoxy)IodoBenzene ( 2.1g; 1.1eq.) were added. After 1 hr, the mixture was diluted with ethyl acetate (100ml) and washed with saturated aqueous sodium hydrogen 25 carbonate (200ml), aqueous potassium hydrogen sulfate (200ml, 1M), and aqueous saturated sodium chloride (200ml). The organic layer was dried over sodium sulfate, filtered and the solvent removed *in vacuo*. Boc-D-(N<sup>i</sup>Boc)Trp-D-g(N<sup>i</sup>Boc)Trp-H was used immediately for the next reaction of formylation.

R<sub>f</sub> = 0.14 { ethyl acetate/hexane (7/3)}.

30 C<sub>36</sub>H<sub>47</sub>N<sub>5</sub>O<sub>7</sub>, 661 g.mol<sup>-1</sup>.

<sup>1</sup>H NMR (200 MHz, DMSO-d<sub>6</sub>) : δ 1.29 (s, 9H, Boc); 1.61 (s, 18H, 2 Boc); 2.13 (s, 2H, NH<sub>2</sub> (amine)); 3.1-2.8 (m, 4H, 2 (CH<sub>2</sub>)<sub>β</sub>); 4.2 (m, 1H, CH<sub>α</sub>); 4.85 (m, 1H, CH<sub>α</sub>); 6.9-8 (m, 12H, 2 indoles (10H), NH (urethane), NH (amide)).

Mass Spectrometry (Electrospray), m/z 662 [M+H]<sup>+</sup>, 684 [M+Na]<sup>+</sup>.

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### Boc-D-(N<sup>t</sup>Boc)Trp-D-g(N<sup>t</sup>Boc)Trp-CHO

Boc-D-(N<sup>t</sup>Boc)Trp-D-g(N<sup>t</sup>Boc)Trp-H (4.3mmol; 1eq.) was dissolved in DMF (20ml). Then, N,N-diisopropylethylamine (815μl; 1.1eq.) and 2,4,5-trichlorophenylformate (1.08g; 1.1eq.) were added. After 30 minutes, the mixture was diluted with ethyl acetate (100ml) and washed with saturated aqueous sodium hydrogen carbonate (200ml), aqueous potassium hydrogen sulfate (200ml, 1M), and saturated aqueous sodium chloride (200ml). The organic layer was dried over sodium sulfate, filtered and the solvent removed *in vacuo*. The residue was purified by flash chromatography on silica gel eluting with ethyl acetate/hexane {5/5} to afford 2.07g of Boc-D-(N<sup>t</sup>Boc)Trp-D-g(N<sup>t</sup>Boc)Trp-CHO as a white solid.

Yield = 70%.

C<sub>37</sub>H<sub>47</sub>N<sub>5</sub>O<sub>8</sub>, 689 g.mol<sup>-1</sup>.

R<sub>f</sub> = 0.27 { ethyl acetate/hexane (5/5)}.

<sup>1</sup>H NMR (200 MHZ, DMSO-d<sub>6</sub>) : δ 1.28 (s, 9H, Boc); 1.6 (s, 9H, Boc); 1.61 (s, 9H, Boc); 2.75-3.1 (m, 4H, 2 (CH<sub>2</sub>)<sub>B</sub>); 4.25 (m, 1H, (CH)α<sub>A & B</sub>); 5.39 (m, 0.4H, (CH)α'<sub>B</sub>); 5.72 (m, 0.6H, (CH)α'<sub>A</sub>); 6.95-8.55 (m, 14H, 2 indoles (10H), NH (urethane), 2 NH (amides), CHO (formyl)).

Mass Spectrometry (Electrospray), m/z 690 [M+H]<sup>+</sup>, 712 [M+Na]<sup>+</sup>, 1379 [2M+H]<sup>+</sup>.

### Boc-Aib-D-Trp-D-gTrp-CHO

Boc-D-(N<sup>t</sup>Boc)Trp-D-g(N<sup>t</sup>Boc)Trp-CHO (1.98g; 2.9mmol; 1eq.) was dissolved in a mixture of trifluoroacetic acid (16ml), anisole (2ml) and thioanisole (2ml) for 30 minutes at 0°C. The solvents were removed *in vacuo*, the residue was stirred with ether and the precipitated TFA, H-D-Trp-D-gTrp-CHO was filtered.

TFA, H-D-Trp-D-gTrp-CHO (2.9mmol; 1eq.), Boc-Aib-OH (700mg; 1eq.), NMM (2.4ml; 4.2eq.) and BOP (1.53g; 1.2eq.) were successively added in 10ml of DMF. After 1 hr, the mixture was diluted with ethyl acetate (100ml) and washed with saturated aqueous sodium hydrogen carbonate (200ml), aqueous potassium hydrogen sulfate (200ml, 1M), and saturated aqueous sodium chloride (200ml). The organic layer was dried over sodium sulfate, filtered and the solvent removed *in vacuo*. The residue was purified by flash chromatography on silica gel eluting with ethyl acetate to afford 1.16g of Boc-Aib-D-Trp-D-gTrp-CHO as a white solid.

Yield = 70%.

C<sub>31</sub>H<sub>38</sub>N<sub>6</sub>O<sub>5</sub>, 574 g.mol<sup>-1</sup>.

R<sub>f</sub> = 0.26 {Chloroform/Methanol/ Acetic Acid (180/10/5)}.

<sup>1</sup>H NMR (200 MHZ, DMSO-d<sub>6</sub>) : δ 1.21 (s, 6H, 2 CH<sub>3</sub>(Aib)); 1.31 (s, 9H, Boc); 2.98-3.12 (m, 4H, 2 (CH<sub>2</sub>)<sub>B</sub>); 4.47 (m, 1H, (CH)α<sub>A & B</sub>); 5.2 (m, 0.4H, (CH)α'<sub>B</sub>); 5.7 (m,

0.6H, (CH) $\alpha'_A$ ); 6.95-8.37 (m, 15H, 2 indoles (10H), 3 NH (amides), 1 NH (urethane), CHO (formyl)); 10.89 (m, 2H, 2 N<sup>1</sup>H (indoles)).

Mass Spectrometry (Electrospray), m/z 575 [M+H]<sup>+</sup>, 597 [M+Na]<sup>+</sup>, 1149 [2M+H]<sup>+</sup>, 1171 [2M+Na]<sup>+</sup>.

5

#### H-Aib-D-Trp-D-gTrp-CHO

Boc-Aib-D-Trp-D-gTrp-CHO (1g; 1.7mmol) was dissolved in a mixture of trifluoroacetic acid (8ml), anisole (1ml) and thioanisole (1ml) for 30 minutes at 0°C. The solvents were removed *in vacuo*, the residue was stirred with ether and the precipitated

10 TFA, H-Aib-D-Trp-D-gTrp-CHO was filtered.

The product TFA, H-Aib-D-Trp-D-gTrp-CHO was purified by preparative HPLC (Waters, delta pak, C18, 40x100mm, 5 $\mu$ m, 100 Å).

Yield = 52%.

C<sub>26</sub>H<sub>30</sub>N<sub>6</sub>O<sub>3</sub>, 474 g.mol<sup>-1</sup>.

15 <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) + <sup>1</sup>H/<sup>1</sup>H correlation :  $\delta$  1.21 (s, 3H, CH<sub>3</sub> (Aib)); 1.43 (s, 3H, CH<sub>3</sub> (Aib)); 2.97 (m, 2H, (CH<sub>2</sub>)<sub>B</sub>); 3.1 (m, 2H, (CH<sub>2</sub>)<sub>B</sub>); 4.62 (m, 1H, (CH) $\alpha_{A\&B}$ ); 5.32 (q, 0.4H, (CH) $\alpha'_B$ ); 5.71 (q, 0.6H, (CH) $\alpha'_A$ ); 7.3 (m, 4H, H<sub>5</sub> and H<sub>6</sub> (2 indoles)); 7.06-7.2 (4d, 2H, H<sub>2A</sub> et H<sub>2B</sub> (2 indoles)); 7.3 (m, 2H, H<sub>4</sub> or H<sub>7</sub> (2 indoles)); 7.6-7.8 (4d, 2H, H<sub>4A</sub> and H<sub>4B</sub> or H<sub>7A</sub> et H<sub>7B</sub>); 7.97 (s, 3H, NH<sub>2</sub> (Aib) and CHO (Formyl)); 8.2 (d, 0.4H, NH<sub>1B</sub> (diamino)); 8.3 (m, 1H, NH<sub>A\&B</sub>); 8.5 (d, 0.6H, NH<sub>1A</sub> (diamino)); 8.69 (d, 0.6H, NH<sub>2A</sub> (diamino)); 8.96 (d, 0.4H, NH<sub>2B</sub> (diamino)); 10.8 (s, 0.6H, N<sup>1</sup>H<sub>1A</sub> (indole)); 10.82 (s, 0.4H, N<sup>1</sup>H<sub>1B</sub> (indole)); 10.86 (s, 0.6H, N<sup>1</sup>H<sub>2A</sub> (indole)); 10.91 (s, 0.4H, N<sup>1</sup>H<sub>2B</sub> (indole)).

25 Mass Spectrometry (Electrospray), m/z 475 [M+H]<sup>+</sup>, 949 [2M+H]<sup>+</sup>.

Analogous synthesis were performed for the following compounds:

#### Example 2 H-Aib-D-Mrp-D-gMrp-CHO

C<sub>28</sub>H<sub>34</sub>N<sub>6</sub>O<sub>3</sub>, 502 g.mol<sup>-1</sup>.

30 <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) + <sup>1</sup>H/<sup>1</sup>H correlation :  $\delta$  1.19 (s, 2H, (CH<sub>3</sub>)<sub>1A</sub> (Aib)); 1.23 (s, 1H, (CH<sub>3</sub>)<sub>1B</sub> (Aib)); 1.41 (s, 2H, (CH<sub>3</sub>)<sub>2A</sub> (Aib)); 1.44 (s, 2H, (CH<sub>3</sub>)<sub>2B</sub> (Aib)); 2.33-2.35 (4s, 6H, 2 CH<sub>3</sub> (indoles)); 2.93 (m, 2H, (CH<sub>2</sub>)<sub>B</sub>); 3.02 (m, 2H, (CH<sub>2</sub>)<sub>B</sub>); 4.65 (m, 0.6H, (CH) $\alpha_A$ ); 4.71 (m, 0.4H, (CH) $\alpha_B$ ); 5.2 (m, 0.4H, (CH) $\alpha'_B$ ); 5.6 (m, 0.6H, (CH) $\alpha'_A$ ); 6.95 (m, 4H, H<sub>5</sub> and H<sub>6</sub> (2 indoles)); 7.19 (m, 2H, H<sub>4</sub> or H<sub>7</sub> (2 indoles)); 7.6 (m, 2H, H<sub>4</sub> or H<sub>7</sub> (2 indoles)); 7.9 (s, 1H, CHO (Formyl)); 7.95 (s, 2H, NH<sub>2</sub> (Aib)); 8.05 (d, 0.4H, NH<sub>1B</sub> (diamino)); 8.3 (m, 1H, NH<sub>A\&B</sub>); 8.35 (m, 0.6H, NH<sub>1A</sub> (diamino)); 8.4 (d, 0.6H, NH<sub>2A</sub>

(diamino)); 8.75 (d, 0.4H, NH<sub>2B</sub> (diamino)); 10.69 (s, 0.6H, N<sup>1</sup>H<sub>1A</sub> (indole)); 10.71 (s, 0.4H, N<sup>1</sup>H<sub>1B</sub> (indole)); 10.80 (s, 0.6H, N<sup>1</sup>H<sub>2A</sub> (indole)); 10.92 (s, 0.4H, N<sup>1</sup>H<sub>2B</sub> (indole)).

Mass Spectrometry (Electrospray), m/z 503.1 [M+H]<sup>+</sup>.

5 **Example 3** N-Me-Aib-D-Trp-D-gTrp-CHO

Boc-N-Me-Aib-OH (327mg; 1.5mmol; 2.6eq.) was dissolved in methylene chloride (10ml) and cooled to 0°C. Then, dicyclohexylcarbodiimide (156mg; 0.75mmol; 1.3eq.) was added. The mixture, after filtration of DCU, was added to a solution containing TFA, H-D-Trp-D-gTrp-CHO (0.58mmol; 1eq.) and triethylamine (267μl; 3.3eq.) in methylene chloride (5ml). The reaction mixture was slowly warmed to room temperature and stopped after 24 hr. The mixture was diluted with ethyl acetate (25ml) and washed with saturated aqueous sodium hydrogen carbonate (50ml), aqueous potassium hydrogen sulfate (50ml, 1M), and saturated aqueous sodium chloride (50ml). The organic layer was dried over sodium sulfate, filtered and the solvent removed *in vacuo*. The residue was purified by flash chromatography on silica gel eluting with ethyl acetate/methanol {9/1} to afford 180mg (53%) of Boc-N-Me-Aib-D-Trp-D-gTrp-CHO as a white foam.

Boc-N-Me-Aib-D-Trp-D-gTrp-CHO (180mg; 0.3mmol) was dissolved in a mixture of trifluoroacetic acid (8ml), anisole (1ml) and thioanisole (1ml) for 30 minutes at 0°C. The solvents were removed *in vacuo*, the residue was stirred with ether and the precipitated TFA, N-Me-Aib-D-Trp-D-gTrp-CHO was filtered.

The product TFA, N-Me-Aib-D-Trp-D-gTrp-CHO (39mg; 15%) was purified by preparative HPLC (Waters, delta pak, C18, 40x100mm, 5μm, 100 Å).

C<sub>27</sub>H<sub>32</sub>N<sub>6</sub>O<sub>3</sub>, 488 g.mol<sup>-1</sup>.

<sup>1</sup>H RMN (200 MHZ, DMSO-d<sub>6</sub>) : δ 1.19 (s, 3H, CH<sub>3</sub> (Aib)); 1.42 (s, 3H, CH<sub>3</sub> (Aib)); 2.26 (s, 3H, NCH<sub>3</sub>); 3.12 (m, 4H, 2 (CH<sub>2</sub>)<sub>B</sub>); 4.66 (m, 1H, (CH)<sub>a</sub>); 5.32 et 5.7 (m, 1H, (CH)<sub>a</sub>); 6.9-7.8 (m, 10H, 2 indoles); 8 (m, 1H, CHO (formyl)); 8.2-9 (m, 4H, 3 NH (amides) et NH (amine)); 10.87 (m, 2H, 2 N<sup>1</sup>H (indoles)).

Mass Spectrometry (Electrospray), m/z 489.29 [M+H]<sup>+</sup>.

30 **Example 4** H-Aib-D-Trp-D-gTrp-C(O)CH<sub>3</sub>

Boc-D-(N<sup>1</sup>Boc)Trp-D-g(N<sup>1</sup>Boc)Trp-H (0.72mmol; 1eq.) was dissolved in DMF (20ml). Then, N,N-diisopropylethylamine (259ml; 2.1eq.) and acetic anhydride (749ml; 1.1eq.) were added. After 1 hr, the mixture was diluted with ethyl acetate (100ml) and washed with saturated aqueous sodium hydrogen carbonate (100ml), aqueous potassium hydrogen sulfate (100ml, 1M), and saturated aqueous sodium chloride (50ml). The organic layer was dried over sodium sulfate, filtered and the solvent removed *in vacuo*. The residue

was purified by flash chromatography on silica gel eluting with ethyl acetate/hexane to afford 370mg (73%) of Boc-D-(N<sup>t</sup>Boc)Trp-D-g(N<sup>t</sup>Boc)Trp-C(O)CH<sub>3</sub> as a white solid.

Boc-D-(N<sup>t</sup>Boc)Trp-D-g(N<sup>t</sup>Boc)Trp-C(O)CH<sub>3</sub> (350mg; 0.5mmol; 1eq.) was dissolved in a mixture of trifluoroacetic acid (8ml), anisole (1ml) and thioanisole (1ml) for 30 minutes at 0°C. The solvents were removed *in vacuo*, the residue was stirred with ether and the precipitated TFA, H-D-Trp-D-gTrp-C(O)CH<sub>3</sub> was filtered.

In 10ml of DMF, TFA, H-D-Trp-D-gTrp-C(O)CH<sub>3</sub> (0.5mmol; 1eq.), Boc-Aib-OH (121mg; 0.59mmol; 1.2eq.), NMM (230μl; 4.2eq.) and BOP (265mg; 1.2eq.) were successively added. After 1 hr, the mixture was diluted with ethyl acetate (25ml) and washed with saturated aqueous sodium hydrogen carbonate (50ml), aqueous potassium hydrogen sulfate (50ml, 1M), and saturated aqueous sodium chloride (50ml). The organic layer was dried over sodium sulfate, filtered and the solvent removed *in vacuo*. The residue was purified by flash chromatography on silica gel eluting with ethyl acetate to afford 249mg (85%) of Boc-Aib-D-Trp-D-gTrp-C(O)CH<sub>3</sub> as a white foam.

Boc-Aib-D-Trp-D-gTrp-C(O)CH<sub>3</sub> (249mg; 0.42mmol) was dissolved in a mixture of trifluoroacetic acid (8ml), anisole (1ml) and thioanisole (1ml) for 30 minutes at 0°C. The solvents were removed *in vacuo*, the residue was stirred with ether and the precipitated TFA, H-Aib-D-Trp-D-gTrp-C(O)CH<sub>3</sub> was filtered.

The product TFA, H-Aib-D-Trp-D-gTrp-C(O)CH<sub>3</sub> (80mg; 23%) was purified by preparative HPLC (Waters, delta pak, C18, 40x100mm, 5mm, 100 Å).

C<sub>27</sub>H<sub>32</sub>N<sub>6</sub>O<sub>3</sub>, 488 g.mol<sup>-1</sup>.

<sup>1</sup>H NMR (200 MHz, DMSO-d<sub>6</sub>) : δ 1.22 (s, 3H, CH<sub>3</sub> (Aib)); 1.44 (s, 3H, CH<sub>3</sub> (Aib)); 1.8 (s, 3H, C(O)CH<sub>3</sub>); 3.06 (m, 4H, 2 (CH<sub>2</sub>)<sub>β</sub>); 4.6 (m, 1H, (CH)<sub>α</sub>); 5.6 (m, 1H, (CH)<sub>α</sub>); 6.9-7.8 (m, 10H, 2 indoles); 7.99 (s, 2H, NH<sub>2</sub> (Aib)); 8.2-8.6 (m, 3H, 3 NH (amides)); 10.83 (s, 2H, 2 N<sup>t</sup>H (indoles)).

Mass Spectrometry (Electrospray), m/z 489.32 [M+H]<sup>+</sup>.

#### Example 5 N-Me-Aib-D-Trp-D-gTrp-C(O)CH<sub>3</sub>

Boc-N-Me-Aib-OH (1.09g; 5.04mmol; 4eq.) was dissolved in methylene chloride (10ml) and cooled to 0°C. Then, dicyclohexylcarbodiimide (520mg; 2.52mmol; 2eq.) was added. The mixture, after filtration of DCU, was added to a solution containing TFA, H-D-Trp-D-gTrp-C(O)CH<sub>3</sub> (940mg; 1.26mmol; 1eq.) and triethylamine (580ml; 3.3eq.) in methylene chloride (5ml). The reaction mixture was slowly warmed to room temperature and stopped after 24 h. The mixture was diluted with ethyl acetate (50ml) and washed with saturated aqueous sodium hydrogen carbonate (100ml), aqueous potassium hydrogen sulfate (100ml, 1M), and saturated aqueous sodium chloride (100ml). The organic layer was dried

over sodium sulfate, filtered and the solvent removed *in vacuo*. The residue was purified by flash chromatography on silica gel eluting with ethyl acetate/methanol {9/1} to afford 530mg (70%) of Boc-N-Me-Aib-D-Trp-D-gTrp-C(O)CH<sub>3</sub> as a white foam.

- 5 Boc-N-Me-Aib-D-Trp-D-gTrp-C(O)CH<sub>3</sub> (530mg; 0.88mmol) was dissolved in a mixture of trifluoroacetic acid (8ml), anisole (1ml) and thioanisole (1ml) for 30 minutes at 0°C. The solvents were removed *in vacuo*, the residue was stirred with ether and the precipitated TFA, N-Me-Aib-D-Trp-D-gTrp-C(O)CH<sub>3</sub> was filtered.

The product TFA, N-Me-Aib-D-Trp-D-gTrp-C(O)CH<sub>3</sub> (220mg; 30%) was purified by preparative HPLC (Waters, delta pak, C18, 40x100mm, 5mm, 100 Å).

- 10 C<sub>26</sub>H<sub>34</sub>N<sub>6</sub>O<sub>3</sub>, 502 g.mol<sup>-1</sup>.

<sup>1</sup>H NMR (200 MHz, DMSO-d<sub>6</sub>) : δ 1.17 (s, 3H, CH<sub>3</sub> (Aib)); 1.4 (s, 3H, CH<sub>3</sub> (Aib)); 1.78 (s, 3H, C(O)CH<sub>3</sub>); 2.23 (s, 3H, NCH<sub>3</sub>); 3.15 (m, 4H, 2 (CH<sub>2</sub>)<sub>β</sub>); 4.7 (m, 1H, (CH)<sub>α</sub>); 5.55 (m, 1H, (CH)<sub>α</sub>); 6.9-7.9 (m, 10H, 2 indoles); 8.2-8.8 (s, 4H, NH (amine) et 3 NH (amides)); 10.8 (s, 2H, 2 N<sup>1</sup>H (indoles)).

- 15 Mass Spectrometry (Electrospray), m/z 503.19 [M+H]<sup>+</sup>.

#### Example 6 Pip-D-Trp-D-gTrp-CHO

In 5ml of DMF, TFA, H-D-Trp-D-gTrp-CHO (230mg; 0.31mmol; 1eq.), Boc-(N<sup>t</sup>Boc)Pip-OH (130mg; 0.38mmol; 1.2eq.), NMM (145μl; 4.2eq.) and BOP (167mg; 0.38mmol; 1.2eq.) were successively added. After 15 minutes, the reaction was over. The mixture was diluted with ethyl acetate (25ml) and washed with saturated aqueous sodium hydrogen carbonate (50ml), aqueous potassium hydrogen sulfate (50ml, 1M), and saturated aqueous sodium chloride (50ml). The organic layer was dried over sodium sulfate, filtered and the solvent removed *in vacuo* to afford Boc-(N<sup>t</sup>Boc)Pip-D-Trp-D-gTrp-CHO as a foam.

- 25 Boc-(N<sup>t</sup>Boc)Pip-D-Trp-D-gTrp-CHO (0.31mmol) was dissolved in a mixture of trifluoroacetic acid (8ml), anisole (1ml) and thioanisole (1ml) for 30 minutes at 0°C. The solvents were removed *in vacuo*, the residue was stirred with ether and TFA, H-Pip-D-Trp-D-gTrp-CHO was filtered.

- 30 The product TFA, H-Pip-D-Trp-D-gTrp-CHO (127mg; 42%) was purified by preparative HPLC (Waters, delta pak, C18, 40x100mm, 5μm, 100 Å).

C<sub>28</sub>H<sub>33</sub>N<sub>7</sub>O<sub>3</sub>, 515 g.mol<sup>-1</sup>.

- <sup>1</sup>H RMN (200 MHz, DMSO-d<sub>6</sub>) : δ 1.81 (m, 2H, CH<sub>2</sub> (Pip)); 2.3 (m, 2H, CH<sub>2</sub> (Pip)); 3.1 (m, 8H, 2 (CH<sub>2</sub>)<sub>β</sub> et 2 CH<sub>2</sub> (Pip)); 4.68 (m, 1H, (CH)<sub>α</sub>); 5.3 et 5.73 (2m, 1H, (CH)<sub>α</sub>); 6.9-7.7 (m, 10H, 2 indoles); 7.98 (2s, 1H, CHO (formyl)); 8.2-9.2 (m, 6H, NH<sub>2</sub> et 3 NH (Pip) et 3 NH (amides)); 10.9 (m, 2H, 2 N<sup>1</sup>H (indoles)).

Mass Spectrometry (Electrospray), m/z 516.37 [M+H]<sup>+</sup>, 538.27 [M+Na]<sup>+</sup>.



Example 7 Pip-D-Trp-D-gTrp-C(O)CH<sub>3</sub>

In 5ml of DMF, TFA, H-D-Trp-D-gTrp-C(O)CH<sub>3</sub> (218mg, 0.29mmol; 1eq.), Boc-(N<sup>t</sup>Boc)Pip-OH (121mg; 0.35mmol; 1.2eq.), NMM (135  $\mu$ l; 4.2eq.) and BOP (155mg; 0.35mmol; 1.2eq.) were successively added. After 15 minutes, the reaction was over. The mixture was diluted with ethyl acetate (25ml) and washed with saturated aqueous sodium hydrogen carbonate (50ml), aqueous potassium hydrogen sulfate (50ml, 1M), and saturated aqueous sodium chloride (50ml). The organic layer was dried over sodium sulfate, filtered and the solvent removed *in vacuo* to afford Boc-(N<sup>t</sup>Boc)Pip-D-Trp-D-gTrp-C(O)CH<sub>3</sub> as a foam.

Boc-(N<sup>t</sup>Boc)Pip-D-Trp-D-gTrp-C(O)CH<sub>3</sub> (0.29mmol) was dissolved in a mixture of trifluoroacetic acid (8ml), anisole (1ml) and thioanisole (1ml) for 30 minutes at 0°C. The solvents were removed *in vacuo*, the residue was stirred with ether and the precipitated TFA, H-Pip-D-Trp-D-gTrp-C(O)CH<sub>3</sub> was filtered.

The product TFA, H-Pip-D-Trp-D-gTrp-C(O)CH<sub>3</sub> (135mg; 47%) was purified by preparative HPLC (Waters, delta pak, C18, 40x100mm, 5 $\mu$ m, 100 Å).

C<sub>29</sub>H<sub>35</sub>N<sub>7</sub>O<sub>3</sub>, 529 g.mol<sup>-1</sup>.

<sup>1</sup>H RMN (200 MHz, DMSO-d<sub>6</sub>) :  $\delta$  1.79 (m, 2H, CH<sub>2</sub> (Pip)); 1.81 (s, 3H, C(O)CH<sub>3</sub>); 2.3 (m, 2H, CH<sub>2</sub> (Pip)); 3.1 (m, 8H, 2 (CH<sub>2</sub>)<sub>6</sub> et 2 CH<sub>2</sub> (Pip)); 4.7 (m, 1H, (CH)  $\alpha$ ); 5.6 (m, 1H, (CH)  $\omega$ ); 6.9-7.8 (m, 10H, 2 indoles); 8.2-9 (m, 6H, NH<sub>2</sub> et NH (Pip) et 3 NH (amides)); 10.85 (m, 2H, 2 N<sup>t</sup>H (indoles)).

Mass Spectrometry (Electrospray), m/z 530.39 [M+H]<sup>+</sup>, 552.41 [M+Na]<sup>+</sup>.

Example 8 Isonipecotyl-D-Trp-D-gTrp-CHO

In 5ml of DMF, TFA, H-D-Trp-D-gTrp-CHO (250mg, 4.1mmol; 1eq.), Fmoc-Isonipecotyl-OH (144mg; 4.1mmol; 1.2eq.), NMM (158 $\mu$ l; 4.2eq.) and BOP (181mg; 4.1mmol; 1.2eq.) were successively added. After 15 minutes, the reaction was over. The mixture was diluted with ethyl acetate (25ml) and washed with saturated aqueous sodium hydrogen carbonate (50ml), aqueous potassium hydrogen sulfate (50ml, 1M), and saturated aqueous sodium chloride (50ml). The organic layer was dried over sodium sulfate, filtered and the solvent removed *in vacuo* to afford Fmoc-Isonipecotyl-D-Trp-D-gTrp-CHO as a foam.

Fmoc-Isonipecotyl-D-Trp-D-gTrp-CHO (4.1mmol) was dissolved in a mixture of DMF (8ml) and piperidine (2ml) and allowed to stand for 30 minutes. The solvents were removed *in vacuo*, the residue was stirred with ether and the precipitated Isonipecotyl-D-Trp-D-gTrp-CHO was filtered.

The product Isonipecotyl-D-Trp-D-gTrp-CHO (81mg; 28%) was purified by preparative HPLC (Waters, delta pak, C18, 40x100mm, 5µm, 100 Å).

$C_{28}H_{32}N_6O_3$ , 500 g.mol<sup>-1</sup>.

- <sup>1</sup>H RMN (200 MHZ, DMSO-d<sub>6</sub>) : δ 1.65 (m, 4H, 2 CH<sub>2</sub> (Pip)); 2.4 (m, 1H, CH (Pip)); 2.7-3.3 (m, 8H, 2 (CH<sub>2</sub>)<sub>β</sub> et 2 CH<sub>2</sub> (Pip)); 4.6 (m, 1H, (CH) <sub>α</sub>); 5.3 et 5.7 (2m, 1H, (CH) <sub>α</sub>); 6.9-7.7 (m, 10H, 2 indoles); 7.97 (2s, 1H, CHO (formyl)); 8-8.8 (m, 4H, NH (Pip) et 3 NH (amides)); 10.9 (m, 2H, 2 N<sup>1</sup>H (indoles)).

Mass Spectrometry (Electrospray), m/z 501.36 [M+H]<sup>+</sup>.

10 Example 9 Isonipecotyl-D-Trp-D-gTrp-C(O)CH<sub>3</sub>

- In 5ml of DMF, TFA, H-D-Trp-D-gTrp-C(O)CH<sub>3</sub> (250mg, 0.33mmol; 1eq.), Fmoc-Isonipectic-OH (141mg; 0.4mmol; 1.2eq.), NMM (155µl; 4.2eq.) and BOP (178mg; 0.4mmol; 1.2eq.) were successively added. After 15 minutes, the reaction was over. The mixture was diluted with ethyl acetate (25ml) and washed with saturated aqueous sodium hydrogen carbonate (50ml), aqueous potassium hydrogen sulfate (50ml, 1M), and saturated aqueous sodium chloride (50ml). The organic layer was dried over sodium sulfate, filtered and the solvent removed *in vacuo* to afford Fmoc-Isonipecotyl-D-Trp-D-gTrp-C(O)CH<sub>3</sub> as a foam.

- Fmoc-Isonipecotyl-D-Trp-D-gTrp-C(O)CH<sub>3</sub> (0.33mmol) was dissolved in a mixture of DMF (8ml) and piperidine (2ml) and allowed to stand for 30 minutes. The solvents were removed *in vacuo*, the residue was stirred with ether and the precipitated Isonipecotyl-D-Trp-D-gTrp-C(O)CH<sub>3</sub> was filtered.

The product Isonipecotyl-D-Trp-D-gTrp-C(O)CH<sub>3</sub> (65mg; 13%) was purified by preparative HPLC (Waters, delta pak, C18, 40x100mm, 5µm, 100 Å).

- 25  $C_{29}H_{34}N_6O_3$ , 514 g.mol<sup>-1</sup>.

<sup>1</sup>H RMN (200 MHZ, DMSO-d<sub>6</sub>) : δ 1.66 (m, 4H, 2 CH<sub>2</sub> (Pip)); 1.79 (s, 3H, C(O)CH<sub>3</sub>); 2.7-3.3 (m, 8H, 2 (CH<sub>2</sub>)<sub>β</sub> et 2 CH<sub>2</sub> (Pip)); 4.54 (m, 1H, (CH) <sub>α</sub>); 5.59 (m, 1H, (CH) <sub>α</sub>); 6.9-7.7 (m, 10H, 2 indoles); 8-8.6 (m, 4H, NH (Pip) et 3 NH (amides)); 10.82 (m, 2H, 2 N<sup>1</sup>H (indoles)).

- 30 Mass Spectrometry (Electrospray), m/z 515.44 [M+H]<sup>+</sup>.

Examples 10-62

The following compounds were prepared in similar manners:

- 35 Example 10 H-Aib-D-Mrp-gMrp-CHO

Example 11 H-Aib-Trp-gTrp-CHO

Example 12 H-Aib-Trp-D-gTrp-CHO

5 Example 13 H-D-Trp-gTrp-CHO

Example 14 N-Me-D-Trp-gTrp-CHO

Example 15 N-Methylsulfonyl-D-Trp-gTrp-CHO

10

Example 16 N-Phenylsulfonyl-D-Trp-gTrp-CHO

Example 17 N-(3-Methyl-butanoyl)-D-Trp-gTrp-CO-CH<sub>3</sub>

15 Example 18 N-(3-Methyl-butanoyl)-D-Trp-gTrp-CHO

Example 19 Aib-D-Trp-gTrp-CO-CH<sub>2</sub>-CH<sub>3</sub>

Example 20 Aib-D-Trp-gTrp-CO-CH<sub>2</sub>-CH(CH<sub>3</sub>)-CH<sub>3</sub>

20

Example 21 Aib-D-Trp-gTrp-CO-CH<sub>2</sub>-phenyl

Example 22 Aib-D-Trp-gTrp-CO-piperidin-4-yl

25 Example 23 Aib-D-Trp-gTrp-CO-CH<sub>2</sub>-pyrrol-3-yl

Example 24 Aib-D-Trp-gTrp-CO-CH<sub>2</sub>-CH<sub>2</sub>-cyclohexyl

Example 25 N-Me-Aib-D-Trp-gTrp-CO-CH<sub>2</sub>-CH<sub>3</sub>

30

Example 26 N-Me-Aib-D-Trp-gTrp-CO-CH<sub>2</sub>-CH(CH<sub>3</sub>)-CH<sub>3</sub>

Example 27 N-Me-Aib-D-Trp-gTrp-CO-CH<sub>2</sub>-phenyl

35 Example 28 N-Me-Aib-D-Trp-gTrp-CO-CH<sub>2</sub>-pyrrol-3-yl

Example 29 N-Me-Aib-D-Trp-gTrp-CO-CH<sub>2</sub>-CH<sub>2</sub>-cyclohexyl

Example 30 Aib-D-Trp-gTrp-CHO

5 Example 31 N-(3-amino-3-methyl-butanoyl)-D-Trp-gTrp-CO-CH<sub>3</sub>

Example 32 N-Acetyl-D-Trp-gTrp-CHO

Example 33 N-Acetyl-D-Trp-gTrp-CO-CH<sub>3</sub>

10

Example 34 N-Formyl-D-Trp-gTrp-CHO

Example 35 N-Formyl-D-Trp-gTrp-CO-CH<sub>3</sub>

15 Example 36 N-(1,1-dimethyl-2-amino-2-keto-ethyl)-D-Trp-gTrp-CHO

Example 37 N-(2-amino-2-methyl-propyl)-D-Trp-gTrp-CHO

Example 38 N-(2-amino-2-methyl-propyl)-D-Trp-gTrp-CO-CH<sub>3</sub>

20

Example 39 N-Me-Aib-D-Trp-D-gTrp-Isonipecotyl

Example 40 N-Me-Aib-D-Trp-N-Me-D-gTrp-C(O)CH<sub>3</sub>

25 Example 41 H-Aib-D-Trp-N-Me-D-gTrp-C(O)CH<sub>3</sub>

Example 42 H-Aib-(D)-1-Nal-g-(D)-1-Nal-formyl

C<sub>30</sub>H<sub>32</sub>N<sub>4</sub>O<sub>3</sub>, 496 g.mol<sup>-1</sup>.

<sup>1</sup>H RMN (200 MHz, DMSO-d<sub>6</sub>): δ 1.14 and 1.4 (2m, 6H, 2 CH<sub>3</sub> (Aib)); 3.17-3.55 (m, 4H, 2 (CH<sub>2</sub>)<sub>β</sub>); 4.82 (m, 1H, CHα); 5.5 and 5.82 (2m, 1H, CHα); 7.36-7.64 (m, 8H); 7.83-8 (m, 7H); 8.25-9.45 (m, 5H).

Mass Spectrometry (FAB), m/z 497 [M+H]<sup>+</sup>.

Analytic HPLC (Delta Pak 5μ C18 100A, 1ml/min, 214nm, eluent: H<sub>2</sub>O / ACN 0.1% TFA, gradient 0 to 100% ACN in 50min), tr = 20.28min, 99%. Freezedried Compound.

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Example 43 H-Aib-(D)-2-Nal-g-(D)-2-Nal-formyl

$C_{30}H_{32}N_4O_3$ , 496 g.mol<sup>-1</sup>.

**<sup>1</sup>H RMN (200 MHz, DMSO-d<sub>6</sub>):** δ 1.18 and 1.36 (2m, 6H, 2 CH<sub>3</sub> (Aib)) ; 2.84-3.3 (m, 4H, 2 (CH<sub>2</sub>)<sub>β</sub>) ; 4.7 (m, 1H, CH<sub>α</sub>); 5.45 and 5.73 (2m, 1H, CH<sub>α</sub>); 7.47-7.51 (m, 6H); 7.76-8.06 (m, 11H); 8.36-9.11 (m, 3H).

5 **Mass Spectrometry (FAB), m/z 497 [M+H]<sup>+</sup>.**

**Analytic HPLC** (Delta Pak 5μ C18 100A, 1ml/min, 214nm, eluent: H<sub>2</sub>O / ACN 0.1% TFA, gradient 0 to 100% ACN in 50min), tr = 20.26min, 95%. Freezedried Compound.

Example 44 H-Aib-(D)-1-Nal-g-(D)-Trp-formyl

10  $C_{28}H_{31}N_5O_3$ , 485 g.mol<sup>-1</sup>.

**<sup>1</sup>H RMN (200 MHz, DMSO-d<sub>6</sub>):** δ 1.15 and 1.42 (2m, 6H, 2 CH<sub>3</sub> (Aib)) ; 3.11-3.3 and 3.54-3.7 (m, 4H, 2 (CH<sub>2</sub>)<sub>β</sub>) ; 4.81 (m, 1H, CH<sub>α</sub>) ; 5.4 and 5.74 (2m, 1H, CH<sub>α</sub>) ; 7.06-7.2 (m, 3H); 7.34-7.65 (m, 6H) ; 7.91-8.1 (m, 4H) ; 8.2-8.4 (m, 1H); 8.55-9.5 (m, 3H) ; 10.95 (m, 1H, N<sup>1</sup>H).

15 **Mass Spectrometry (FAB), m/z 486 [M+H]<sup>+</sup>.**

**Analytic HPLC** (Delta Pak 5μ C18 100A, 1ml/min, 214nm, eluent: H<sub>2</sub>O / ACN 0.1% TFA, gradient 0 to 100% ACN in 50min), tr = 17.33min, 92%. Freezedried Compound.

Example 45 H-Aib-(D)-2-Nal-g-(D)-Trp-formyl

20  $C_{28}H_{31}N_5O_3$ , 485 g.mol<sup>-1</sup>.

**<sup>1</sup>H RMN (200 MHz, DMSO-d<sub>6</sub>):** δ 1.19 and 1.45 (2m, 6H, 2 CH<sub>3</sub> (Aib)) ; 2.93-3.3 (m, 4H, 2 (CH<sub>2</sub>)<sub>β</sub>) ; 4.71 (m, 1H, CH<sub>α</sub>) ; 5.35 and 5.7 (2m, 1H, CH<sub>α</sub>) ; 7.05-7.1 (m, 2H); 7.2-7.34 (m, 1H) ; 7.47-7.53 (m, 4H) ; 7.64 (m, 1H) ; 7.78-8 (m, 8H); 8.48-9.37 (m, 2H); 10.88-11.04 (m, 1H, N<sup>1</sup>H).

25 **Mass Spectrometry (FAB), m/z 486 [M+H]<sup>+</sup>.**

**Analytic HPLC** (Delta Pak 5μ C18 100A, 1ml/min, 214nm, eluent: H<sub>2</sub>O / ACN 0.1% TFA, gradient 0 to 100% ACN in 50min), tr = 17.30min, 95%. Freezedried Compound.

Example 46 H-Aib-(D)-Trp-g-(D)-1-Nal-formyl

30  $C_{28}H_{31}N_5O_3$ , 485 g.mol<sup>-1</sup>.

**<sup>1</sup>H RMN (200 MHz, DMSO-d<sub>6</sub>):** δ 1.23 and 1.41 (2m, 6H, 2 CH<sub>3</sub> (Aib)) ; 2.92-3.15 (m, 2H, (CH<sub>2</sub>)<sub>β</sub>) ; 3.4-3.6 (m, 2H, (CH<sub>2</sub>)<sub>β</sub>) ; 4.63 (m, 1H, CH<sub>α</sub>) ; 5.44 and 5.79 (2m, 1H, CH<sub>α</sub>) ; 6.99-7.15 (m, 3H) ; 7.33 (m, 1H) ; 7.45-8.1 (m, 11H); 8.34-9.37 (m, 3H); 10.83 (m, 1H).

**Mass Spectrometry (FAB), m/z 486 [M+H]<sup>+</sup>.**

35 **Analytic HPLC** (Symmetry shield 3.5μ C18 100A, 1ml/min, 214nm, eluent: H<sub>2</sub>O / ACN

0.1% TFA, gradient 0 to 60% ACN in 15min then 60 to 100% ACN in 3min), tr = 10.00min, 99%. Freezedried Compound.

**Example 47** H-Aib-D-Trp-g-D-2-Nal-formyl

- 5  $C_{28}H_{31}N_5O_3$ , 485 g.mol<sup>-1</sup>.

**<sup>1</sup>H RMN (200 MHz, DMSO-d<sub>6</sub>)**: δ 1.22 and 1.43 (2m, 6H, 2 CH<sub>3</sub> (Aib)); 2.85-3.3 (m, 4H, 2 (CH<sub>2</sub>)<sub>6</sub>); 4.64 (m, 1H, CH<sub>2</sub>); 5.37 and 5.72 (2m, 1H, CH<sub>2</sub>); 6.97-7.13 (m, 3H); 7.32 (m, 1H); 7.44-7.54 (m, 3H); 7.66 (d, 1H); 7.78 (m, 1H); 7.86-8.02 (m, 7H); 8.33-9.4 (m, 2H); 10.82 (m, 1H, N<sup>1</sup>H).

- 10 **Mass Spectrometry (FAB)**, m/z 486 [M+H]<sup>+</sup>.

**Analytic HPLC** (Delta pak 5μ C18 100A, 1ml/min, 214nm, eluent: H<sub>2</sub>O / ACN 0.1% TFA, gradient 0 to 100% ACN in 25min), tr = 9.00min, 99%. Freezedried Compound.

**Example 48** H-Aib-(D)-Trp-g-(D)-3-(R/S)Dht-formyl

- 15  $C_{26}H_{32}N_6O_3$ , 476 g.mol<sup>-1</sup>.

**<sup>1</sup>H RMN (400 MHz, DMSO-d<sub>6</sub>)**: δ 1.12 (s, 3H, CH<sub>3</sub> (Aib)); 1.32 (s, 3H, CH<sub>3</sub> (Aib)); 1.73 (m, 1H, CH<sub>2</sub>); 2.01 (m, 1H, CH<sub>2</sub>); 2.9 (m, 1H); 3.03 (m, 1H); 3.13 (m, 2H); 3.54 (m, 1H); 4.47 (m, 1H, CH<sub>2</sub>); 5.10 and 5.52 (2m, 1H, CH<sub>2</sub>); 6.71-8.83 (m, 16H, 5H (Trp), 4H (Dht), 3 NH (amides), NH and NH<sub>2</sub> (amines), formyl); 10.7 (m, 1H, N<sup>1</sup>H).

- 20 **Mass Spectrometry (Electrospray)**, m/z 477.46 [M+H]<sup>+</sup> 499.42 [M+Na]<sup>+</sup>; 953.51 [2M+H]<sup>+</sup>.

**Analytic HPLC** (Delta Pak 5μ C18 100A, 1ml/min, 214nm, eluent: H<sub>2</sub>O / ACN 0.1% TFA, gradient 0 to 100% ACN in 50min), tr = 9.40min, 98%. Freezedried Compound.

- 25 **Example 49** H-Aib-(D)-3(R/S)Dht-g-(D)-Trp-formyl

$C_{26}H_{32}N_6O_3$ , 476 g.mol<sup>-1</sup>.

**RMN <sup>1</sup>H(400 MHz, DMSO-d<sub>6</sub>)**: δ 1.58 (s, 3H, CH<sub>3</sub> (Aib)); 1.85 (m, 1H, CH<sub>2</sub>); 2.2 (m, 1H, CH<sub>2</sub>); 3.1 (d, 2H); 3.35 (m, 2H); 3.56 (m, 1H); 3.7 (m, 1H); 4.5 (m, 1H, CH<sub>2</sub>); 5.33 and 5.71 (2m, 1H, CH<sub>2</sub>); 6.88-8.91 (m, 16H, 5H (Trp), 4H (Dht), 3 NH (amides), NH and NH<sub>2</sub> (amines), formyl); 10.92 and 10.97 (2s, 1H, N<sup>1</sup>H).

- 30 **Mass Spectrometry (Electrospray)**, m/z 477.33 [M+H]<sup>+</sup>; 499.42 [M+Na]<sup>+</sup> 953.51 [2M+H]<sup>+</sup>.

**Analytic HPLC** (Delta Pak 5μ C18 100A, 1ml/min, 214nm, eluent: H<sub>2</sub>O / ACN 0.1% TFA, gradient 0 to 100% ACN in 50min), tr = 10.35min, 98%. Freezedried Compound.

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**Example 50** N-Me-Aib-(D)-Trp-g-(D)-3(R/S)Dht-acetyl

$C_{28}H_{36}N_6O_3$ , 504 g.mol<sup>-1</sup>.

**<sup>1</sup>H RMN (400 MHz, DMSO-d<sup>6</sup>):** δ 1.42 (s, 3H, CH<sub>3</sub> (Aib)); 1.63 (s, 3H, CH<sub>3</sub> (Aib)); 2.72 (m, 3H, acetyl); 2.4 (m, 2H, CH<sub>2</sub>); 2.5 (m, 3H, NCH<sub>3</sub>); 3.2-3.5 (m, 4H); 3.85 (m, 1H); 4.85 (m, 1H, CH<sub>2</sub>); 5.76 (m, 1H, CH<sub>2</sub>); 7.04-8.86 (m, 14H, 5H (Trp), 4H (Dht), 3 NH (amides),

5 2 NH (amines)); 11.02 (2s, 1H, N<sup>1</sup>H).

**Mass Spectrometry (Electrospray),** m/z 505,31 [M+H]<sup>+</sup>; 527,70 [M+Na]<sup>+</sup>.

**Analytic HPLC** (Delta Pak 5μ C18 100A, 1ml/min, 214nm, eluent : H<sub>2</sub>O / ACN 0.1% TFA, gradient 0 to 100% ACN in 50min), tr = 10.20min, 98%. Freezedried Compound.

10 **Example 51** N-Me-Aib-(D)-3(R/S)Dht-g-(D)-Trp-acetyl

$C_{28}H_{36}N_6O_3$ , 504 g.mol<sup>-1</sup>.

**<sup>1</sup>H RMN (400 MHz, DMSO-d<sup>6</sup>):** δ 1.58 (s, 6H, 2 CH<sub>3</sub> (Aib)); 1.81 (m, 3H, acetyl); 1.98 (m, 1H, CH<sub>2</sub>); 2.24 (m, 1H, CH<sub>2</sub>); 2.54 (m, 3H, NCH<sub>3</sub>); 3.08 (d, 2H); 3.31 (m, 2H); 3.4 (m, 1H); 3.59 (m, 1H); 3.71 (m, 1H); 4.52 (m, 1H, CH<sub>2</sub>); 5.61 (m, 1H, CH<sub>2</sub>); 6.9-8.92 (m,

15 14H, 5H (Trp), 4H (Dht), 3 NH (amides), 2 NH (amines)); 10.88 (s, 1H, N<sup>1</sup>H).

**Mass Spectrometry (Electrospray),** m/z 505.43 [M+H]<sup>+</sup>; 527.52 [M+Na]<sup>+</sup>.

**Analytic HPLC** (Delta Pak 5μ C18100A, 1ml/min, 214nm, eluent : H<sub>2</sub>O / ACN 0.1% TFA, gradient 0 to 100% ACN in 50min), tr = 11 min, 98%. Freezedried Compound.

20 **Example 52** N(Me)<sub>2</sub>-Aib-(D)-Trp-(D)-gTrp-formyl

$C_{28}H_{36}N_6O_3$ , 502 g.mol<sup>-1</sup>.

**<sup>1</sup>H RMN (400 MHz, DMSO-d<sup>6</sup>):** δ 1.2 (s, 3H, CH<sub>3</sub> (Aib)); 1.39 (s, 3H, CH<sub>3</sub> (Aib)); 2.29 (m, 3H, NCH<sub>3</sub>); 2.99-3.33 (m, 4H, 2 (CH<sub>2</sub>)<sub>p</sub>); 4.68 (m, 1H, CH<sub>2</sub>); 5.3 and 5.69 (m, 1H, CH<sub>2</sub>); 6.97-7.72 (m, 10H, 2 indoles); 7.97 (2s, 1H, formyl); 8.2-9.47 (m, 3H, 3 NH

25 (amides)); 10.85 (m, 2H, 2 NH (indoles)).

**Mass Spectrometry (Electrospray),** m/z 503.45 [M+H]<sup>+</sup>.

**Analytic HPLC** (Symmetry shield 3.5μ C18 100A, 1ml/min, 214nm, eluent : H<sub>2</sub>O / ACN 0.1% TFA, gradient 0 to 100% ACN in 15min), tr = 6.63 min, 99%. Freezedried Compound.

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**Example 53** N(Me)<sub>2</sub>-Aib-D-Trp-D-gTrp-acetyl

$C_{29}H_{36}N_6O_3$ , 516 g.mol<sup>-1</sup>.

**<sup>1</sup>H RMN (200 MHz, DMSO-d<sup>6</sup>):** δ 1.22 (s, 3H, CH<sub>3</sub> (Aib)); 1.4 (s, 3H, CH<sub>3</sub> (Aib)); 1.8 (s, 3H, acetyl); 2.28 (d, 3H, NCH<sub>3</sub>); 2.96-3.22 (m, 4H, 2 (CH<sub>2</sub>)<sub>p</sub>); 4.7 (m, 1H, (CH<sub>2</sub>)<sub>2</sub>); 5.60

35 (m, 1H, (CH<sub>2</sub>)<sub>2</sub>); 6.98-7.75 (m, 10H, 2 indoles); 8.2-9.47 (m, 3H, 3 NH (amides)); 10.84 (m, 2H, 2 NH (indoles)).

**Mass Spectrometry (Electrospray),**  $m/z$  517.34  $[M+H]^+$ .

**Analytic HPLC** (Symmetry shield 3.5 $\mu$  C18 100A, 1ml/min, 214nm, eluent: H<sub>2</sub>O / ACN 0.1% TFA, gradient 0 to 100% ACN in 15min),  $tr$  = 7.07min, 99%. Freezedried Compound.

5 **Example 54** H-Acc<sup>3</sup>-(D)-Trp-(D)-gTrp-formyl

C<sub>26</sub>H<sub>28</sub>N<sub>6</sub>O<sub>3</sub>, 472 g.mol<sup>-1</sup>.

**<sup>1</sup>H RMN (400 MHz, DMSO-d<sub>6</sub>)** :  $\delta$  1.11 and 1.5 (2m, 4H, 2 CH<sub>2</sub> (Acc<sup>3</sup>)) ; 2.91-3.12 (m, 4H,

2 (CH<sub>2</sub>)<sub>6</sub>) ; 4.6 (m, 1H, CH<sub>2</sub>) ; 5.3 and 5.7 (2m, 1H, CH<sub>2</sub>) ; 6.97-7.17 (m, 6H, indoles) ; 7.32

10 (m, 2H, indoles) ; 7.62-7.72 (m, 2H, indoles) ; 7.97 (2s, 1H, formyl) ; 8.27-8.92 (m, 5H, 3 NH (amides) and NH<sub>2</sub> (amine)) ; 10.80-10.90 (4s, 2H, 2 N<sup>1</sup>H).

**Mass Spectrometry (Electrospray),**  $m/z$  473.22  $[M+H]^+$  ; 495.15  $[M+Na]^+$  945.47  $[2M+H]^+$  ; 967.32  $[2M+Na]^+$ .

**Analytic HPLC** (Delta Pak 5 $\mu$  C18 100A, 1ml/min, 214nm, eluent : H<sub>2</sub>O / ACN 0.1%

15 TFA, gradient 0 to 100% ACN in 50min),  $tr$  = 14.20min, 98%. Freezedried Compound.

**Example 55** H-Acc<sup>5</sup>-(D)-Trp-(D)-gTrp-formyl

C<sub>26</sub>H<sub>28</sub>N<sub>6</sub>O<sub>3</sub>, 472 g.mol<sup>-1</sup>.

**<sup>1</sup>H RMN (400 MHz, DMSO-d<sub>6</sub>)** :  $\delta$  1.51 and 2.31 (m, 8H, 4 CH<sub>2</sub> (Acc<sup>5</sup>)) ; 2.97-3.18 (m,

20 4H, 2 (CH<sub>2</sub>)<sub>6</sub>) ; 4.64 (m, 1H, CH<sub>2</sub>) ; 5.31 and 5.69 (2m, 1H, CH<sub>2</sub>) ; 6.96-7.34 (m, 8H, indoles) ; 7.62-7.74 (m, 2H, indoles) ; 7.96 (m, 3H, formyl and NH<sub>2</sub> (amine)) ; 8.48-8.96 (m, 3H, 3 NH (amides) ; 10.80-10.90 (4s, 2H, 2 N<sup>1</sup>H).

**Mass Spectrometry (Electrospray),**  $m/z$  501.31  $[M+H]^+$  ; 523.42  $[M+Na]^+$  ; 101.37  $[2M+H]^+$ .

25 **Analytic HPLC** (Delta Pak 5 $\mu$  C18 100A, 1ml/min, 214nm, eluent : H<sub>2</sub>O / ACN 0.1% TFA, gradient 0 to 100% ACN in 50min),  $tr$  = 15.35min, 98%. Freezedried Compound.

**Example 56** H-Acc<sup>6</sup>-(D)-Trp-(D)-gTrp-formyl

C<sub>26</sub>H<sub>28</sub>N<sub>6</sub>O<sub>3</sub>, 472 g.mol<sup>-1</sup>.

30 **<sup>1</sup>H RMN (400 MHz, DMSO-d<sub>6</sub>)** :  $\delta$  1.29-1.57 (m, 8H, 4 CH<sub>2</sub> (Acc<sup>6</sup>)) ; 1.89 and 2.04 (2m, 2H, CH<sub>2</sub> (Acc<sup>6</sup>)) ; 2.95-3.17 (m, 4H, 2 (CH<sub>2</sub>)<sub>6</sub>) ; 4.61 (m, 1H, CH<sub>2</sub>) ; 5.3 and 5.68 (2m, 1H, CH<sub>2</sub>) ; 6.95-7.21 (m, 6H, indoles) ; 7.32 (m, 2H, indoles) ; 7.6 (m, 2H, indoles) ; 7.74 (m, 2H, indoles) ; 7.96 (m, 3H, formyl and NH<sub>2</sub> (amine)) ; 8.18-8.67 (m, 5H, 3 NH (amides)) ; 10.77-10.89 (4s, 2H, 2N<sup>1</sup>H).

35 **Mass Spectrometry (Electrospray),**  $m/z$  515.11  $[M+H]^+$ .



**Analytic HPLC** (Delta Pak 5 $\mu$  C18 100A, 1ml/min, 214nm, eluent : H<sub>2</sub>O / ACN 0.1% TFA, gradient 0 to 100% ACN in 50min), tr = 15.9min, 97%. Freezedried Compound.

Example 57 H-Dpg-(D)-Trp-(D)-gTrp-formyl

5 C<sub>26</sub>H<sub>28</sub>N<sub>6</sub>O<sub>3</sub>, 530 g.mol<sup>-1</sup>.

<sup>1</sup>H RMN (400 MHz, DMSO-d<sub>6</sub>) :  $\delta$  0 (m, 1H, Dpg) ; 0.40 (m, 3H, Dpg) ; 0.70 (m, 4H, Dpg) ; 1.01-1.51 (m, 5H, Dpg) ; 1.76 (m, 1H, Dpg) ; 2.82-2.95 (m, 4H, 2 (CH<sub>2</sub>)<sub>6</sub>) ; 4.59 (m, 1H, CH<sub>2</sub>) ; 5.3 and 5.54 (2m, 1H, CH<sub>2</sub>) ; 6.81-7.09 (m, 6H, indoles) ; 7.19 (m, 2H, indoles) ; 7.48 (m, 1H, indoles) ; 7.6-7.68 (m, 5H, 1H (indoles), formyl and NH<sub>2</sub> (amine) ; 7.83-8.82 (m, 3H, 3 NH (amides)) ; 10.69 and 10.76 (2m, 2H, 2N<sup>1</sup>H).

**Mass Spectrometry (Electrospray)**, m/z 531.24 [M+I]<sup>+</sup>.

**Analytic HPLC** (Delta Pak 5 $\mu$  C18 100A, 1ml/min, 214nm, eluent : H<sub>2</sub>O / ACN 0.1% TFA, gradient 0 to 100% ACN in 50mm), tr = 15.35mm, 98%. Freezedried Compound.

15 Example 58 H-Aib-(D)-Trp-(D)-gTrp-C(O)NHCH<sub>2</sub>CH<sub>3</sub>

C<sub>26</sub>H<sub>25</sub>N<sub>7</sub>O<sub>3</sub>, 517 g.mol<sup>-1</sup>.

<sup>1</sup>H RMN (400 MHz, DMSO-d<sub>6</sub>) :  $\delta$  0.94 (t, 3H, NHCH<sub>2</sub>CH<sub>3</sub>) ; 1.01 (s, 3H, CH<sub>3</sub> (Aib)) ; 1.08 (s, 3H, CH<sub>3</sub> (Aib)) ; 1.8 (sl, 2H, NH<sub>2</sub>) ; 2.95-3.15 (m, 6H, 2 (CH<sub>2</sub>)<sub>6</sub> and NHCH<sub>2</sub>CH<sub>3</sub>) ; 4.43 (m, 1H, CH<sub>2</sub>) ; 5.39 (m, 1H, CH<sub>2</sub>) ; 6.02 (m, 1H) ; 6.22 (m, 1H) ; 6.9-7.56 (m, 10H, indoles) ; 8 (m, 1H) ; 8.31 (m, 1H) ; 10.77 and 10.79 (2s, 2H, 2N<sup>1</sup>H).

**Mass Spectrometry (Electrospray)**, m/z 518.4 [M+H]<sup>+</sup> ; 540.3 [M+Na]<sup>+</sup>.

**Analytic HPLC** (Symmetry shield 3.5 $\mu$  C18 100A, 1ml/min, 214nm, eluent : H<sub>2</sub>O / ACN 0.1% TFA, gradient 0 to 100% ACN in 15min), tr = 7.12min, 99%. Freezedried Compound.

25 Example 59 N-Me-Aib-(D)-Trp-(D)-gTrp-C(O)NHCH<sub>2</sub>CH<sub>3</sub>

Example 60 H-Aib-(R)-Me-Trp-(D)-gTrp-formyl

Example 61 H-Aib-(D)-Trp-(R)-Me-gTrp-formyl

30

Example 62 H-Me-Aib-(D)-Trp-(R)-Me-gTrp-acetyl

Example 63 EVALUATION OF THE GROWTH HORMONE RELEASING  
ACTIVITY OF NEW GROWTH HORMONE SECRETAGOGUES  
IN THE INFANT RAT

35

### Animals

Male 10-day-old Sprague Dawley rats, about 25 g body weight were used.

Pups were received on the fifth day after birth and were housed under controlled conditions ( $22 \pm 2^\circ\text{C}$ , 65 % humidity and artificial light from 06.00 to 20.00 h). A standard dry diet and water were available ad libitum to the dams.

### Experimental procedure

One hour before the experiments, pups were separated from their respective dams and were divided randomly into groups of eight each.

Pups were acutely challenged subcutaneously with 100  $\mu\text{l}$  of solvent (DMSO, final dilution 1:300 in physiological saline), hexarelin (Tyr-Ala-His-D-Mrp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>, used as a reference drug), or new compounds (300  $\mu\text{g/kg}$ ) and killed by decapitation 15 min later.

Trunk blood was collected and centrifuged immediately. Plasma samples were stored at  $-20^\circ\text{C}$  until assayed for the determination of plasma GH concentrations.

Growth hormone concentrations in plasma were measured by RIA using materials provided by the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) of the National Institute of Health U.S.A.

Values were expressed in terms of the NIDDK-rat-GH-RP-2 standard (potency 2IU/mg) as ng/ml of plasma.

The minimum detectable value of rat GH was about 1.0 ng/ml, and intraassay variability was about 6 %.

The obtained results of several test series, wherein the in vivo activity in the rat was determined, are listed in tables 1 to 10.

Table 1

Example	Structure	GH ng/ml
1	H-Aib-D-Trp-D-gTrp-CHO	$158.8 \pm 39.4$
13	H-Aib-D-Trp-gTrp-CHO	$58 \pm 6.3$
SOLVENT		$15.0 \pm 8.0$
HEXARELIN	Tyr-Ala-His-D-Mrp-Ala-Trp-D-Phe-Lys-NH <sub>2</sub>	$202 \pm 32.7$

Table 2

Example	Structure	GH ng/ml
3	N-Me-Aib-D-Trp-D-gTrp-CHO	86.6 ± 12.6
4	H-Aib-D-Trp-D-gTrp-C(O)CH <sub>3</sub>	104.7 ± 13.5
5	N-Me-Aib-D-Trp-D-gTrp-C(O)CH <sub>3</sub>	175.5 ± 37.2
SOLVENT		20.7 ± 0.9
HEXARELIN	Tyr-Ala-His-D-Mrp-Ala-Trp-D-Phe-Lys-NH <sub>2</sub>	134.5 ± 27.2

Table 3

Example	Structure	GH ng/ml
6	Pip-D-Trp-D-gTrp-CHO	109.7 ± 10.1
7	Pip-D-Trp-D-gTrp-C(O)CH <sub>3</sub>	53.1 ± 6.6
8	Isonipecotyl-D-Trp-D-gTrp-CHO	94.2 ± 8.6
9	Isonipecotyl-D-Trp-D-gTrp-C(O)CH <sub>3</sub>	61.2 ± 10.8
19	Aib-D-Trp-gTrp-CO-CH <sub>2</sub> -CH <sub>3</sub>	79.8 ± 22.4
20	Aib-D-Trp-gTrp-CO-Piperidin-4-yl	153.6 ± 30.6
SOLVENT		22.3 ± 5
HEXARELIN	Tyr-Ala-His-D-Mrp-Ala-Trp-D-Phe-Lys-NH <sub>2</sub>	114.7 ± 8.4

Table 4

Example	Structure	GH ng/ml
39	N-Me-Aib-D-Trp-D-gTrp- Isonipecotyl	97.1 ± 21.0
40	N-Me-Aib-D-Trp-N-Me-D-gTrp-C(O)CH <sub>3</sub>	188.2 ± 28.5
41	H-Aib-D-Trp-N-Me-D-gTrp-C(O)CH <sub>3</sub>	75.4 ± 15.0
SOLVENT		10.55 ± 2.65
HEXARELIN	Tyr-Ala-His-D-Mrp-Ala-Trp-D-Phe-Lys-NH <sub>2</sub>	114.5 ± 12.9

Table 5

Example	Structure	GH ng/ml
42	H-Aib-(D)-1-Nal-g-(D)-1-Nal-formyl	25.05 ± 06.00
43	H-Aib-(D)-2-Nal-g-(D)-2-Nal-formyl	37.33 ± 19.74
44	H-Aib-(D)-1-Nal-g-(D)-1-Trp-formyl	15.04 ± 03.30
45	H-Aib-(D)-2-Nal-g-(D)-1-Trp-formyl	13.91 ± 03.87
46	H-Aib-(D)-Trp-g-(D)-1-Nal-formyl	8.26 ± 01.09
47	H-Aib-(D)-Trp-g-(D)-2-Nal-formyl	9.04 ± 04.03
SOLVENT		6.49 ± 01.18
HEXARELIN		276.01 ± 23.5

Table 6

Example	Structure	GH ng/ml
48	H-Aib-(D)-Trp-g-3(R/S)Dht-formyl	17.49 ± 2.40
49	H-Aib-(D)-3(R/S)Dht-(D)-Trp-formyl	24.35 ± 4.85
50	N-Me-Aib-(D)-Trp-(D)-3(R/S)Dht-acetyl	11.17 ± 1.35
51	H-Me-Aib-(D)-3(R/S)Dht-(D)-Trp-acetyl	19.38 ± 4.16
SOLVENT		14.65 ± 0.92
HEXARELIN		91.61 ± 4.09

Table 7

Example	Structure	GH ng/ml
52	N(Me) <sub>2</sub> -Aib-(D)-Trp-(D)-gTrp-formyl	121.43 ± 29
53	N(Me) <sub>2</sub> -Aib-(D)-Trp-(D)-gTrp-acetyl	26.80 ± 5.64
SOLVENT		7.89 ± 1.77
HEXARELIN		172.5 ± 38.53

Table 8

Example	Structure	GH ng/ml
60	H-Aib-(R)-Me-Trp-(D)-gTrp-formyl	21.02 ± 3.43
61	H-Aib-(D)-Trp-(R)-Me-gTrp-formyl	152.28 ± 43.76
62	H-Me-Aib-(D)-Trp-(R)-Me-gTrp-acetyl	171.78 ± 10.32
SOLVENT		7.89 ± 1.77
HEXARELIN		172.5 ± 38.53

Table 9

Example	Structure	GH ng/ml
54	H-Acc <sup>3</sup> -(D)-Trp-(D)-gTrp-formyl	7.89 ± 3.20
55	H-Acc <sup>5</sup> -(D)-Trp-(D)-gTrp-formyl	11.46 ± 1.18
56	H-Acc <sup>6</sup> -(D)-Trp-(D)-gTrp-formyl	8.49 ± 0.40
57	H-Dpg-(D)-Trp-(D)-gTrp-formyl	18.38 ± 2.88
SOLVENT		17.32 ± 1.70
HEXARELIN		89.91 ± 3.04

Table 10

Example	Structure	GH ng/ml
58	H-Aib-(D)-Trp-(D)-gTrp-C(O)NHCH <sub>2</sub> CH <sub>3</sub>	376.48 ± 43.24
59	N-Me-Aib-(D)-Trp-(D)-gTrp-C(O)NHCH <sub>2</sub> CH <sub>3</sub>	179.53 ± 24.65
SOLVENT		7.89 ± 1.77
HEXARELIN		172.5 ± 38.53

Furthermore the time dependence of the oral activity in the dog (1mg/kg; per os) was estimated for example 1 (H-Aib-D-Trp-D-gTrp-CHO). Well-trained beagles of either sex, > 10 year, 10-15 kg by weight, were used. Animals were fed normal dry food with water ad libitum and were on a 12h-light/12h-dark regimen with on at 7.00. The compound was administered orally to the dogs which had fasted since 16.00 of the preceding day.

Blood samples were taken 20 min before administration, at administration and 15, 30, 60, 90, 120 and 180 min after administration. The results are given in table 11.

Table 11

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Examp e	NAME OF THE DOG							
1	DAKOTA	JORMA	RAZ DEGAN	FORREST LEE	MARKUS	TAYLOR	MEAN VALUE	SEM
t (min)	Concentration GH (ng/ml)							
-20	0.48	3.58	2.14	1.43	2.45	2.32	2.07	0.38
0	0.35	2.75	1.64	2.01	2.55	1.41	1.79	1.03
15	2.11	8.91	3.58	6.38	6.11	4.8	5.32	1.02
30	0.54	6.85	6.37	8.48	6.9	3.89	5.5	1.07
60	0.17	2.65	3.02	4.41	6.51	4.34	3.52	0.84
90	0.4	2.47	2.61	6.42	5.18	4.43	3.59	0.66
120	3.58	2.48	1.94	3.71	4.54	4.28	3.42	0.38
180	3.46	2.82	1.49	3.18	4.12	3.18	3.04	0.36
AUC	328.53	658.38	510.64	888.91	944.26	721.34	675.35	94.47

SEM = Standard deviation

AUC = Area under the curve

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